CLINAL PATTERNS IN FROND ANATOMY OF POLYPODIUM

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Variability in frond anatomy of *Polypodium californicum* Kaulf. has been known as early as Eaton's (1879) monograph delineating two varieties, var. *kaulfussii* D. C. Eaton with coriaceous fronds and var. *intermedium* (H. & A.) D. C. Eaton, a more herbaceous inland form. He reported the former growing usually on rocks near the sea and stated: "The texture of fronds from inland localities is rather thinner than in *P. vulgare,* and the veins are more easily seen; but plants from the seacoast . . . have a firmer frond, and less conspicuous veins," the latter, var. *kaulfussii,* "runs by gradation into the inland form." Kendall (1923) presented evidence for retention of the varieties but stated that the two forms vary within the same morphological limits except for the extremely leathery texture of var. *kaulfussii.* Lloyd (1962) found frond texture to change from herbaceous to coriaceous as a function of distance from the ocean.

Although the phenomenon of anatomical plasticity in relation to the environment has been frequently dealt with, few studies have shown it to occur in a clinal pattern and even fewer studies have offered quantitative anatomical data to demonstrate the actual nature of this change. Shields (1950) summarizes much of the work which has been done relating specific influences of the habitat to morphology. This paper presents an analysis of five populations of *P. californicum* growing at Pt. Reyes, California.

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METHODS AND MATERIALS. Fronds were collected in the field at five locations (A–E. *Lloyd 567, 568, 569, 570, 571,* RSA) during January 1961, and the longest pair of pinnae were killed and fixed in formalin-aceto-alcohol (Johansen, 1940). Material was infiltrated, embedded, and sectioned at 12–15 μ according to the usual paraffin techniques. Sections were stained with Northen's modification of Foster's method (Johansen, 1940) and mounted in Canada Balsam. Surface characters were measured on preparations made by infiltrating and mounting 10 mm squares of dried material in water. Observations were made from transverse sections of pinnae on both cells and tissues to determine variation in each and provide evidence for the nature of anatomical distinctions.

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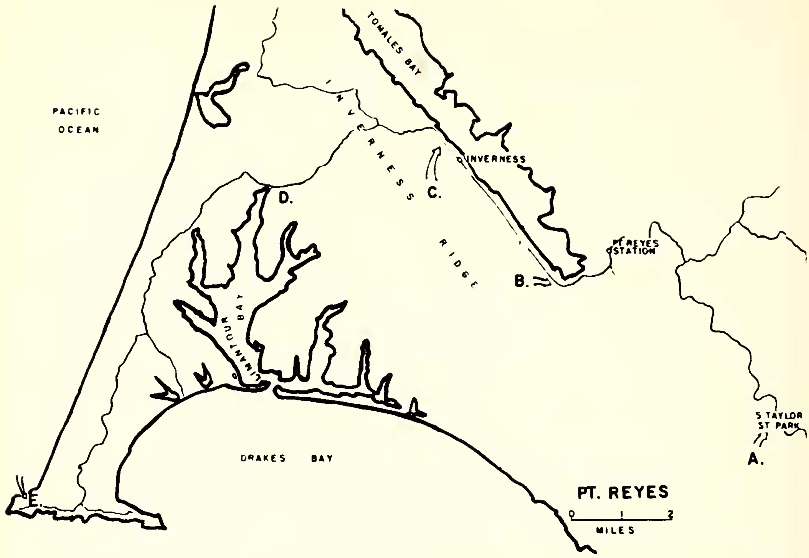


FIG. 1. Map of the Pt. Reyes region showing locations of populations A to E.

ECOLOGY. Pt. Reyes Peninsula, extending 12 miles from the mainland of Marin Co., California, is directly exposed to ocean influences on two sides (fig. 1). Forested areas in more protected regions are gradually replaced by coastal scrub and grassland communities closer to the shore. This change in vegetation is accompanied by higher wind velocity and relative humidity. The topographic relief in relation to the prevailing wind thus defines a mosaic of micro-habitats, but one that changes to greater exposure as the wind assumes dominance over the topography near the coast.

Fronds of *P. californicum* are drought-deciduous unless ground water is available. The summer period, therefore, during which the wind velocity and relative humidity are at their highest on the coast, will have little or no affect on frond characteristics.

The five populations dealt with in this paper are summarized below:

Population A. Fronds of *P. californicum* were collected 2.2 miles east of the entrance to Samuel P. Taylor State Park on a protected road-bank under deep shade of *Sequoia sempervirens*. This location is characteristic of many forested areas of the region in which large herbaceous fronds develop.

Population B. Plants from this location, 1.4 miles west of Pt. Reyes Station on California highway 1, were restricted mainly to moss-covered tree trunks in the moderate shade of *Umbellularia californica* and *Pseudotsuga menziesii* and were in close proximity to Tomales Bay.

Population C. This population, 0.9 miles west of Inverness, is from a habitat similar to but more open than that of population B. The same dominant trees are present. *Polypodium californicum* was found growing both epiphytically and in soil in a predominantly fern understory in moderate shade.

Population D. Plants were collected 5.3 miles west of Inverness by the side of the road to the Pt. Reyes lighthouse. In this area there are no trees and the ferns were found on moss-covered rocks of a north-facing road bank, shaded only part of the day. Associated species were *Mimulus guttatus, Scrophularia californica, Polystichum munitum, Stachys,* and *Rubus.*

Population E. Just north of the lighthouse gate at the southern tip of Pt. Reyes Peninsula, *P. californicum* is found on top of the bluffs above the ocean growing in full sun, only around and under small shrubs of *Vaccinium ovatum* and *Gaultheria shallon.* These species, along with *Lupinus arboreus,* are the dominant shrubs in the coastal grassland. Ferns were usually found on the leeward side of the shrubs where they were partially protected from ocean winds.

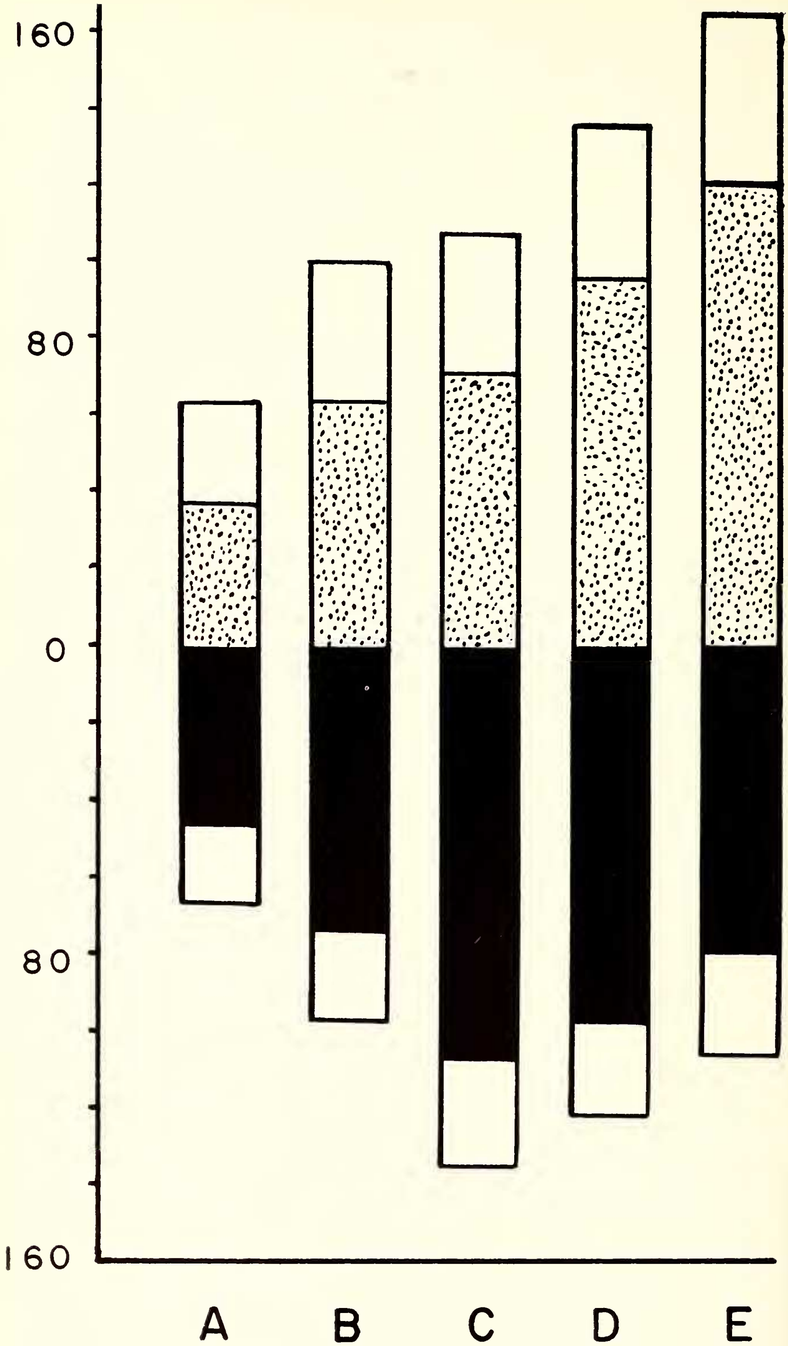
A related species, *P. scouleri* Hook. & Grev., is very rare at this locality but is found in abundance at lower elevations on the bluffs. There is some evidence of hybridization between this species and *P. californicum* in these latter localities (Lloyd, 1962; 1964).

RESULTS. Data from this study are summarized in Tables 1 and 2.

Fronds of *Polypodium* are bifacial but there is no great difference in cell size and shape in the palisade and spongy tissues as is the case in many mesomorphic dicotyledonous plants. For this reason it is sometimes difficult to demarcate tissues. In transverse section, the abaxial cells of the spongy layer are arranged with their long axes parallel with the frond surface. Many air spaces are present. In contrast, the adaxial cells or the palisade layer are very compactly arranged. In both layers there are fewer intercellular spaces near veins, producing a centric arrangement of cells in the chlorenchyma. Virtually all of the cells of the epidermis and mesophyll, except those of the vascular system, contain chlorophyll. Stomata are confined to the abaxial surface of the frond. Trichomes occur abundantly on the latter surface and are bicellular, unbranched, and glandular.

Investigation of fronds has demonstrated a gradient in thickness from a minimum of 128 μ in population A to a maximum of 260 μ in population E. The change in proportion of the various tissues due to increased exposure follows a well established pattern reported in higher plants (Turner, 1923; Weaver and Clements, 1929).

Computation of relative proportions of the tissues of an entire frond shows an increase in the palisade layer as the coast is approached (table 1). There is a corresponding increase in the spongy layer until a maximum is reached at population C (fig. 2) although the mean size of the spongy tissue is larger than the palisade in all populations except


160
80
0
80
160
A
B
C
D
E

E. Epidermal tissue show a range (A–E) from 20 to 13% on the adaxial cells and 14 to 10% on the abaxial side accompanying the grades of the mean thickness of the palisade. However, investigation of individual cell size of epidermal layers shows that both increase in depth and diameter in direct relation to coastal influences (table 2). This is most apparent on the adaxial side which is usually more directly exposed to sun and wind. In both cases the most striking increase is in the diameter of the cells.

Depth of individual cells in the palisade mesophyll gradually increases from a mean of 25 μ in population A to 32 μ in E. The diameters of these cells show a corresponding but greater increase from 31 μ at A to 51 μ at E. In both instances, however, the largest cells observed were in population D. Cell depth of the spongy mesophyll increases from a mean of 20 μ in population A to 28 μ in E. Diameters indicate a much more marked change with an average increase of 26 μ from population A to E. In both layers, but especially in the spongy mesophyll, an obvious decrease in the proportion of intercellular spaces characterize plants from near the coast. Related to this is the percentage of mesophyll cells contiguous with the epidermis. Palisade cells show an increase from 86% to 99.7% from population A to E whereas spongy mesophyll increases more markedly from 56% to 84%.

The number of tiers in the palisade tissue increases from two in the fronds of population A to four or more in population E, and the average thickness of the tissue increases from 37 μ to 120 μ (figs. 3–7). The spongy mesophyll increases in average thickness from 46 μ in population A to 107 μ at C. However, in contrast to palisade mesophyll the spongy tissue decreases in depth to 79 μ in the most xeric population.

Stomatal size remains relatively constant in all populations. The smallest occur in population E. Number of stomata per mm^2, however, varies considerably with a decrease from 33 to 16 in populations A to D and a sharp increase in E to 55. The number of trichomes per mm^2 shows a corresponding decrease in number to population D with a sharp increase at E.

DISCUSSION AND CONCLUSIONS. The populations examined in this paper were chosen for their location in relation to the coast. Habitat characteristics of each relate to the topography of Pt. Reyes, which has a central spine of mountains sloping to low plains at the bluffs above the ocean. Anatomical data can be correlated with these habitats. The basic environmental factors influencing frond histology of the populations studied are probably sunlight and wind exposure. The effect of salt-spray on population E is more pronounced than at the other stations and may help explain the anomalies of increased density of stomata

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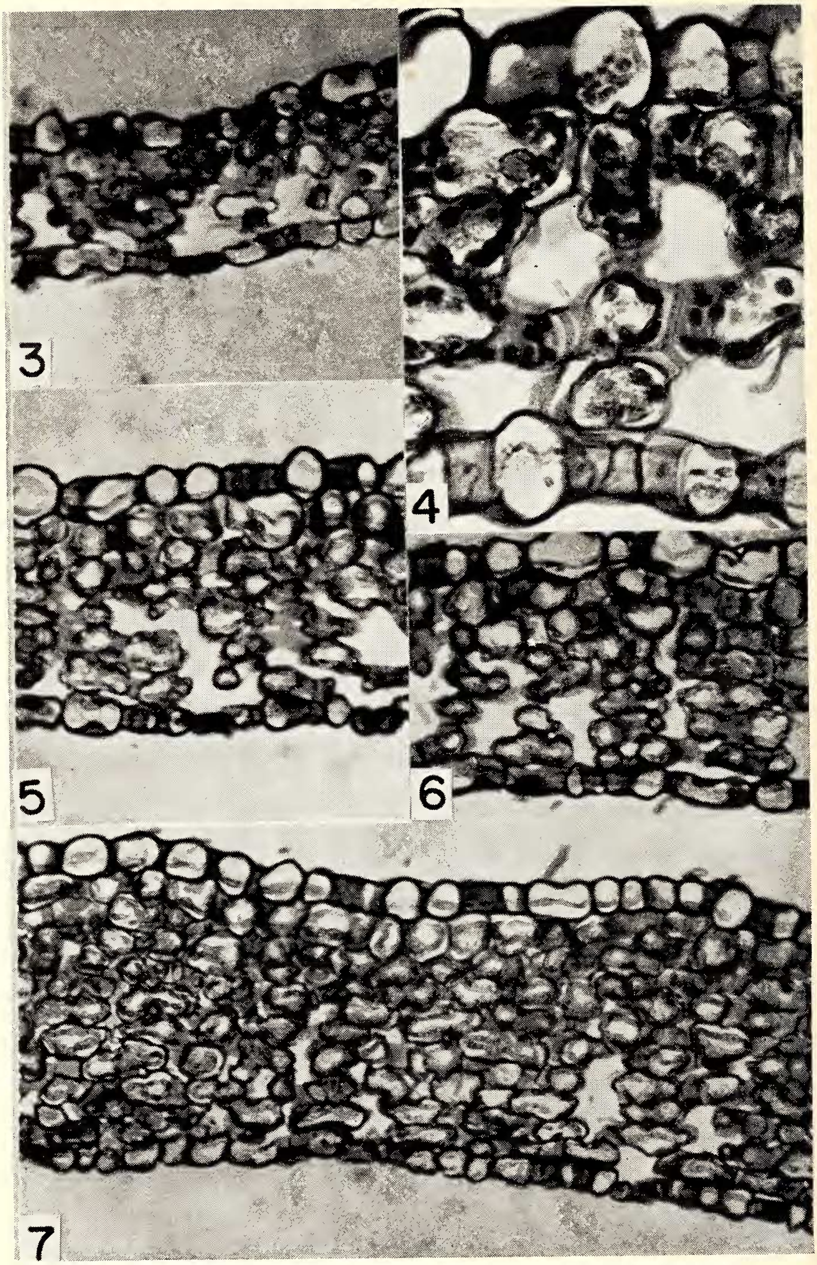
FIG. 2. Composition of the four tissues of the blade; clear = adaxial epidermis; stippled = palisade layer; solid = spongy layer; clear (lower) = abaxial epidermis; abscissa = population; ordinate = number of μ in 20 μ separations.

TABLE 1. AVERAGE SIZE AND RANGE OF FROND TISSUES

Character	Population A	B	C	D	E
Blade length (mm)	258.7	134.0	144.2	119.3	142.6
Blade thickness (μ)	128.0	192.0	240.0	256.0	260.0
range	105–175	145–240	187–318	188-335	190–320
Per cent composition tissues:					
Epidermis, adaxial	20	19	16	15	13
Palisade mesophyll	30	32	30	37	47
Spongy mesophyll	36	37	42	38	30
Epidermis, abaxial	14	12	12	10	10
Tissue thickness (μ)					
Palisade mesophyll	37	64	71	95	120
range	22–55	(32)45–83(125)	50–100	(55)65–125	(70)110–140(175)
Spongy mesophyll	46	73	107	97	79
range	30–75	(30)55–100	65–145(170)	(30)50–132(175)	(35)60–110
Contiguousness with epidermis:					
Palisade (%)	86.0	99.4	97.5	99.7	99.7
Spongy (%	56.0	74.0	67.0	79.0	84.0
No. trichomes/mm²	4	1	0.9	0.75	2.77
No. stomata/mm²	33	30	16	16	55.25
range	24–46	24–36	10–22	(8)12–24	46–62
Stomatal length (μ)	51	67	58	66	51
range	49–60	60–75	45–70	(50)60–75	45–60
Stomatal width (μ)	39	47	46	43	33
range	35–45	42–51	40–52	40–50	25–40
No. fronds examined	31	17	18	29	19

TABLE 2. AVERAGE CELL SIZE AND RANGE WITHIN EACH FROND TISSUE

Tissue	Population A	B	C	D	E
Epidermis:					
adaxial: depth (μ)	26	37	37	41	44
range	17–35	20–52	25–50	23–55	25–55
diameter (μ)	44	51	54	71	58
range	(14)35–90	(20)32–80(105)	(20)30–120	23–120	20–100
abaxial: depth (μ)	20	24	28	25	27
range	11–35	17–37	20–40(65)	15–35	20–40
diameter (μ)	33	44	47	59	56
range	(15)25–60	20–70	20–85(110)	25–95(122)	20–95(120)
Palisade:					
depth (μ)	25	38	32	41	32
range	17–40(50)	20–47	23–50	22–75	15–50(60)
diameter (μ)	31	39	50	58	51
range	13–55(70)	20–50(65)	25–75(125)	33–95	(18)30–95
no. tiers	2.0	2.4	2.5	3.2	4.4
range	1–3	1–3	1–3	2–4	3–7
air space (%)	6.6	0.6	2.5	0.5	0.3
Spongy:					
depth (μ)	20	32	28	33	28
range	15–33	24–42	20–45	20–47	15–40(50)
diameter (μ)	40	55	55	67	66
range	(19)30–65	(33)40–80	20–85(105)	30–90(175)	20–100(115)
air space (%)	45	26	33	21	16



FIGS. 3–7. Transverse section of frond pinnae from populations A to E at Pt. Reyes; 3. Pop. A; 4. Pop. B; 5. Pop. C; 6. Pop. D; 7. Pop. E. Figs. 3, 5–7 × 150; fig. 4 × 450.

and trichomes in that population. However, in the latter case there were more trichomes in population A than E.

In other parts of the range of *P. californicum* plants with herbaceous to semi-coriaceous fronds can be found growing in shaded forests directly above the ocean. Coriaceous fronds have also been found on plants in exposed situations completely removed from coastal influences. Fronds from these latter areas, however, do not reach the extremes in morphology demonstrated by Pt. Reyes plants.

Population A grows in the most mesic environment of all those studied here, a fact clearly reflected in the relatively thin fronds which have thick spongy mesophyll and numerous air spaces. Habitats of populations B and C were open on the north and had less canopy cover. Although they appeared somewhat similar in exposure frond thickness of plants from population C differs from that of B almost to the same extent as B does from A. Contrastingly, differences between plants of C, D, and E are less extreme with a gradual increase in thickness toward the latter. Fronds of population E are more than twice as thick as those of A, a difference attributable to the palisade mesophyll.

Angiosperms may exhibit elongation of palisade cells (Clements, 1905) but this does not seem to be the case in *Polypodium* where the isodiametric cells only become larger. However, in both an increasing amount of palisade tissue forms at the expense of air space and spongy mesophyll. Although individual cells become larger in *Polypodium* with relation to more xeric habitats the increase of tissue thickness results from an increase in cell and tier number. This parallels the situation in *Rumex acetosella* (Transeau, 1904) which exhibits one cell layer of palisade mesophyll in mesic habitats and 2 to 3 layers in xeric environments.

The increase in stomata number in angiosperms inhabiting xeric environments has been noted by Salisbury (1927) and Philpott (1956). This increase in number is usually attributed to spacing and change of epidermal size. It has also been shown that numerous stomata usually have a higher transpiration rate. The sudden increase in stomata from population D to E indicates a drastic change in environment, perhaps regulated by the concentration of salt-spray in the atmosphere. Kunkel (1961) reported larger stomata in fronds of *P. lasiopus* from shaded areas, smaller ones in plants of sunny areas. In addition, fronds more exposed to sun were much more fleshy and approached xerotypes in succulence. He stated that all possible transitional forms may be encountered in individual habitats and feels that both of the forms (shaded and sunny) could be described as ecotypes. Transplant experiments with *P. californicum* have shown that in some instances the coriaceous character of fronds is genetically controlled.

It is evident at Pt. Reyes that there is a clinal gradient in frond structure correlated with increased exposure to sun and wind. Generally, the trends which were discovered may be summarized as follows:

1. There is a direct relationship between frond thickness and degree of habitat modification from mesic to xeric environments.

2. This thickness is due mainly to a greater size of the palisade tissue although corresponding increases accompanied it in other tissues.

3. Individual cell sizes within each tissue was greatest in population D, somewhat smaller in E. The feature responsible for the greater thickness of fronds in the latter was the additional tiers in the palisade mesophyll.

4. Stoma number gradually decreases from mesic to more xeric habitats but sharply increases at the coast.

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